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Appropiate ethical approval was obtained for use of human saphenous vein explant VSMC cultures. This work was supported by The Wellcome Trust, British Heart Foundation and the Garfield-Weston Foundation.

Skeletal muscle sarcoplasmic reticulum (SR) function during altered redox balance using hydrogen peroxide

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Reactive oxygen radicals are produced by skeletal muscle during exercise (Powers *et al.* 1999). Failure to remove these oxidants may result in cellular damage and/or an altered capability to develop tension. Recent evidence *in vitro* suggests maximal force is modulated by altered redox states (Plant *et al.* 2001). However, the mechanism by which this occurs is not fully understood. Likely targets for oxygen radicals include membrane systems and contractile elements, with all receiving little research attention during oxidising conditions. Therefore, the aim of this study was to investigate the effects of altered redox balance on skeletal muscle SR function.

Thirty male Wistar rats were used in this study. Anaesthetised animals (100 mg kg^{-1} sodium pentobarbitone; I.P.) were killed by cervical dislocation, following which the hindlimb muscles extensor digitorum longus (EDL) and soleus were removed. Muscles were incubated in Krebs *with* (Treated, TR) or *without* (Control, CON) 5 mM H_2O_2 for 0, 10, 20, 30 or 40 min. Six ($n = 6$) EDL and soleus muscles were used at each time point for both groups. Following incubation, assays for SR Ca^{2+} uptake and release were performed using previously published methods (Warmington *et al.* 1996).

Results show no difference in SR Ca^{2+} release at all time points (in nM min^{-1} mg^{-1}) between Control and Treated groups in both the EDL (127 ± 8 CON *versus* 117 ± 12 TR at $t = 10$) and soleus (65 ± 9 CON *versus* 85 ± 11 TR at $t = 10$). SR Ca^{2+} uptake was unchanged by H_2O_2 incubation at all time points in soleus (21.5 ± 3 CON *versus* 20 ± 4.2 TR at $t = 10$), although at 20, 30 and 40 min Treated groups showed a significantly reduced rate of SR Ca^{2+} uptake (74.5 ± 6.4 TR at $t = 40$) compared with Control groups in the EDL (99.9 ± 11.2 CON at $t = 40$) ($P < 0.05$, ANOVA).

These data suggest SR function is little affected by oxidative stress. Thus changes in muscle tension induced by oxygen radicals are more likely to arise via actions on other cell components such as the contractile apparatus, rather than alterations in calcium cycling during contraction. However, it seems likely, particularly in fast-twitch muscle (EDL), that a reduction in SR Ca^{2+} uptake following exposure to an oxidative stress may contribute to increased cellular damage or altered force generation during exercise.

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This work was supported by The University of Dublin, Provost Academic Development Fund. The Committee for Protection of Animals used for Scientific/Medical Experiments, Department of Health and Children, Ireland, approved.

The effect of a glycogen phosphorylase inhibitor upon muscle fatigue in anaesthetised rats

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Glycogen phosphorylase (GP) controls glycogenolysis which can be key for muscle contraction both during the initial phase and at high work rates (> 60% $V_{O_2,max}$) (Parolin *et al.* 1999). In the present study we have investigated the effects on inhibition of GP by ZM399527 (5-chloro-*N*-[2-(4-fluorophenyl)-1-(4-hydroxypiperidino carbonyl) ethyl]-1H-indole-2-carboxamide) upon muscle fatigue.

Anaesthetised Wistar rats (alfaxalone/alfadalone, 12 mg kg^{-1} h^{-1}, I.V., $n = 16$) had electrodes placed on the sciatic nerve to induce isometric contraction of the extensor digitorum longus muscle (EDL) (stimulated for 10 min at 2 Hz, 10 ms, supramaximal voltage). Muscle force (set initially at 5 g) was measured 30 min following either the GP inhibitor (ZM399527, 10 mg kg^{-1} I.V.) or vehicle (10% hydroxypropyl β cyclodextrin/0.2% saline). At 10 min of contraction both the contracting and contralateral muscles were removed, and frozen immediately in liquid nitrogen before storing at −80°C prior to analysis. The animals were killed humanely with an overdose of pentobarbitone whilst under anaesthesia,

in accordance with UK regulations. Samples were digested in 1 M KOH for the measurement of tissue glycogen (amyloglucosidase method) and lactate (lactate oxidase method). Concentration of ZM399527 in ethyl acetate extracts of KOH digest of the gastrocnemius muscle was measured by HPLC analysis.

Muscle homogenate concentration of ZM399527 was 15.0 ± 1.3 μM (mean $\pm$ S.E.M.). IC_{50} for the compound on GP is 83 nM (Hoover *et al.* 1998). Peak EDL tension was not different in the two groups (28.8 ± 3.0 and 26.3 ± 0.8 g following vehicle and ZM399527, respectively; Student's *t* test for paired data). In addition, the time to peak tension was unaltered (160 ± 30 *vs.* 113 ± 15 s). Femoral blood flow responses (measured by Doppler flow probe) both at rest (0.00 ± 0.08 *vs.* -0.02 ± 0.21 ml min^{-1}) and during hindlimb contraction (1.58 ± 0.28 *vs.* 1.95 ± 0.16 ml min^{-1}) were not different between vehicle and ZM399527-treated animals. However, EDL fatigue rate was lower (1.02 ± 0.18 and 0.54 ± 0.06 g min^{-1}, $P < 0.03$) and lactate content of the contracting muscle lower in the ZM399527 group (233.2 ± 26.0 *vs.* 164.2 ± 16.9 nmol g^{-1}, $P < 0.05$). Muscle glycogen content after contraction was unaltered by ZM399527 treatment (21 ± 3 *vs.* 19 ± 4 mmol g^{-1}, n.s.).

These data show that at concentrations of GP inhibitor equivalent to 180-fold higher than required for inhibition of muscle GP isoform, lactate formation and muscle fatigue were reduced during muscle contraction. These data therefore suggest that muscle glycogen phosphorylase is not normally a rate-limiting factor in the ability of muscle to maintain work output in the present protocol.

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Exercise induces interleukin-6-dependent genes within human skeletal muscle

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Plasma interleukin (IL)-6 is highly increased in response to strenuous exercise and the production has been shown to originate from the contracting muscles (Pedersen *et al.* 2001). However, the exercise-related stimuli and the purpose of this production are at present unknown. The loss of glycogen may play a role in the production (Steensberg *et al.* 2001). The role of muscle-derived IL-6 could be systemic, but another possibility is that the muscle-derived IL-6 has a local function within the muscles. To look into this possibility, we have investigated whether the expression of IL-6-dependent target genes in the muscle is induced by exercise.

This study investigated the expression of two direct target genes C/EBPβ and C/EBPδ, encoding transcription factors, in the IL-6 signal transduction pathway after exercise. The hypothesis is that the mRNA level for C/EBPβ and C/EBPδ would increase as a result of activation of the IL-6 signal transduction pathway within the muscle.

Muscle biopsies from vastus lateralis were obtained from eight males before, during (1.5 h) and in the hours after running (0, 0.5, 1, 2 and 4 h) at 75% of $V_{O_2,max}$ on a treadmill for 2.5 h. Two control subjects that did not perform exercise were also included. The experimental protocol was approved by the ethics committee and all subjects were informed of the risks and purposes of the study before their written consent was obtained. mRNA for IL-6, C/EBPβ and C/EBPδ were quantified by Northern blotting.

In the exercised muscles C/EBPβ mRNA increased 2-fold at the first time point and remained at that level. C/EBPδ mRNA increased to a 10- to 15-fold elevated level and stayed there until the last time point at 4 h where it dropped to about 2-fold. However, the C/EBPβ mRNA increased in a similar manner in the control subject, whereas only during exercise C/EBPδ mRNA increased to a similar level. At 0–2 h after exercise the increase in C/EBPδ mRNA level was clearly less in the controls. When we looked at the IL-6 mRNA a large increase (more than 10-fold) was seen in the biopsies taken during exercise. A similar increase was seen in one of the controls whereas the other displayed no increase. 0–2 h after exercise the IL-6 mRNA level was 2- to 4-fold increased and returned to basal level at 4 h. The controls remained below a 2-fold increase at these time points.

The data suggest that the sampling procedure itself is influencing the result. The same hole was used twice for taking biopsies and the largest increases in control subjects were seen in the second biopsies. It appears that the invasive action of the biopsy caused production of IL-6 in the local area. However, whereas the increase in C/EBPβ could be explained by the biopsies, the C/EBPδ demonstrated a clear additional exercise effect in the hours after exercise.

In conclusion, the data indicate that the IL-6 signal can be propagated in skeletal muscle, suggesting that the exercise-related IL-6 production represents a local signal within the muscle.